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THE EPIDERMAL CELLS OF ROOTS

A DISSERTATION

SUBMITTED TO THE FACULTY OF THE OGDEN GRADUATE SCHOOL
OF SCIENCE IN CANDIDACY FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

(DEPARTMENT OF BOTANY)

BY

EDITH ADELAIDE ROBERTS

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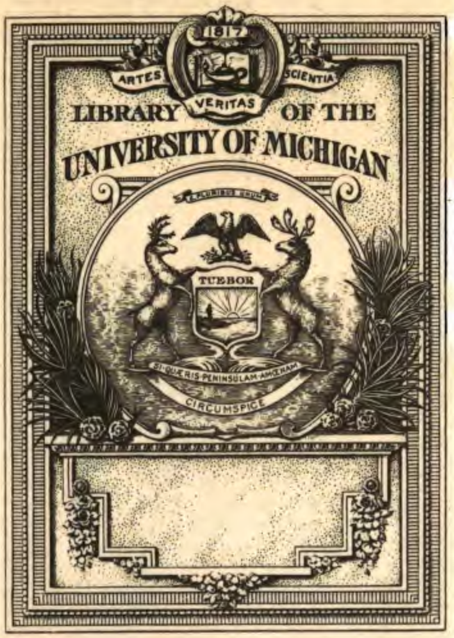
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THE EPIDERMAL CELLS OF ROOTS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 221

EDITH ADELAIDE ROBERTS

(WITH SEVENTEEN FIGURES)

Introduction

The epidermal cells of roots have received more or less attention, especially those epidermal cells which form root hairs. The lines along which investigations have been conducted may be grouped readily under investigations made when the root is in an air medium and those made when the root is in a liquid medium. The factors in the air medium which have been discussed are moisture, light, temperature, contact, length of cells, mode of succession, position of nucleus, osmotic pressure, membranes, and food supply. The factors in the liquid medium discussed are calcium nitrate, potassium nitrate, salt, and bog conditions.

MOISTURE.—PERSECKE (16), working with *Zea mais* and *Pisum sativum*, states that the root hair development depends upon the amount of air and water in the interstices of the soil. SCHWARZ (20), using the same forms, comes to the conclusion that there is a minimum of moisture at which the hair formation begins, an optimum in which the best development is obtained, and a maximum where the hair development nearly or entirely ceases. PFEFFER (18) and others attribute more importance to moisture than to light as a factor in hair development. The conclusion from these observations is that moisture is a factor in the determination of the formation or non-formation of an epidermal cell of a root into an outgrowth called a root hair, but how or why this is so receives no consideration, nor do the limits of the amount of moisture necessary to become a limiting factor.

LIGHT.—The effect of light upon cells in general was investigated by KRAUS (14), who found that darkness increased the length of cells. DEVAUX (2) found the same and that this favored

the development of root hairs on corn. On the other hand, EWART (5), in the formation of root hairs on the root tendrils of *Vanilla*, found darkness accelerating and light retarding their formation. In this work, however, the moisture factor was not eliminated. PETHYBRIDGE (17) found that light retarded the formation of hairs upon oat and corn roots grown in water cultures. SCHWARZ (20) found that light and darkness had no effect on the root hair formation. SNOW (21) states that the effect of light and darkness, if any, is indirect. Light, then, as a factor has been experimented with to some extent with varying results, possibly owing to the fact that it has been associated with other factors.

TEMPERATURE.—No work on temperature as a single factor has been carried on. SCHWARZ (20) found that a temperature of 27–28°C. did not overcome the inhibitory effect of water as the roots grew smooth. SNOW (21), working with high temperatures plus moisture, found a decreased hair production brought about by the increasing elongation of the internal cells.

CONTACT.—The effect of contact has received some attention. SCHWARZ (20) observed that water roots upon entering the substratum develop hairs, and that when the soil is saturated the hairs on corn seedlings disappear, although soil particles are still present, and suggests that it may be due to chemical stimuli or retardation of growth, since it could not be due to contact. PFEFFER (18) denies that contact is a factor, for he found that on climbing roots hairs were produced on the side near the support where there is the greater moisture. SNOW (21) grew corn seedlings between plates and found no hairs on the sides touching the plates, while there were hairs on the other two sides; but again moisture may be the limiting factor and not contact.

JUEL (11) associated short cells and root hairs, as also did VAN TIEGHEM (25) and KRAEMER (13), and he finds that short cells remain short if they do not form root hairs. LEAVITT (15) finds two types of potential root hair cells. The first is that in which any cell of the outer layer may acquire the character of a root hair (trichome) by putting out a hair. This is characteristic of the dicotyledons, of some divisions of the monocotyledons, and of the Filicales. The second type is that in which they originate as

specialized elements. These cells differ in the formation of their cell plate, the wall lies somewhat diagonally, and the cell differs from the other epidermal cells in shape, size, and content. This type he finds in the Schizaeaceae, *Equisetum*, *Azolla*, *Lycopodium*, *Phylloglossum*, *Isoetes*, *Selaginella*, and in Nymphaeaceae. SNOW (21) finds that no definite length can be given as the limit for the formation for hair development; that in some roots the average length of piliferous cells is less than that of the smooth cells, but that the differential elongation of the epidermal and cortical cells is important, and that hair formation depends upon their ratio, that is, between the capacity of the epidermal cells to elongate and their ability to do so.

BARDELL (1) comes to the same conclusion as SNOW. A few of the measurements given by BARDELL are shown in table I.

TABLE I

Plant	Length cortical cell (mm.)	Haired epidermal cell (mm.)	Hairless epidermal cell (mm.)
Pisum sativum......	231	161 (−71)	191 (−40)
" "	179	139 (−40)	172 (− 7)
Zea mais...........	69	66 (− 3)	77 (+ 8)
" "	69	69 =	77 (+ 8)
" "	40	55 (+15)	55 (+15)
" "	36	o	33 (3)
" "	124	58	62

It is difficult to draw the conclusion which is given, for in the one case a difference of 40 mm. in length between epidermal and cortical cells calls forth a root hair; while in another the same difference accounts for the absence of a root hair, and measurements equal to, or greater than, or less than, seem to account for haired or unhaired epidermal cells.

SUCCESSION.—SCHWARZ (20) found that hairs were always produced in acropetal succession, while DEVAUX (2) asserted that new hairs could appear among the old ones; whereas HABERLANDT (8) agrees with SCHWARZ (20), emphasizing the fact that no new hairs ever arise among existing ones.

POSITION OF THE NUCLEUS.—HABERLANDT (8) noted the position of the nucleus in *Brassica alba* to be at the tip. In *Pisum*

sativum he finds that the protrusion of the root hair takes place opposite the nucleus. KÜSTER (12) takes exception to this. In the figures in SNOW'S (21) work the nucleus has no definite position.

OSMOTIC PRESSURE.—PFEFFER (18) found the osmotic pressure in corn root hairs to be greater than that of the cortical cells. STIEHR (23) found that root hairs on seedlings of *Spergula* which had grown in moist air when put in a 1 per cent magnesium solution burst at once, the nucleus being thrown out, and that always a greater percentage of the younger hairs burst. GANONG (7) found that the root hairs of *Salicornia* withstood 90 per cent salt water, *Suaeda maritima* 60–70 per cent, and *Atriplex patulum* 40 per cent. DRABBLE and LAKE (3) found that in mesophyll cells in plants growing in the same condition the osmotic pressure of the cell sap is generally the same, and in the plants of any area the osmotic pressure varies with the physiological scarcity of water. FITTING (6), using cells from the leaf, found that species showing high pressures in dry desert conditions show much lower pressures in moist situations, and concluded that certain plants adjust their osmotic pressure to the medium. ECKERSON (4) found that the root hairs were plasmolyzed by sucrose, varying from 0.20 μ to 0.30 μ. STANGE (22) found that in water cultures with nutrient solutions of high concentrations the osmotic pressures of roots are much higher than others; bean and pea in moist soil have pressures of 6.25N KNO_3 when growing in concentrated medium.

MEMBRANES.—SCHWARZ (20) found that the membrane of the root hair of *Taxus baccata* was of two parts: an inner layer which stains blue with chlorzinc iodide, and an outer layer which stains yellow brown. This outer layer is a mucilage which is hard in dry soil, and as moisture increases strongly swells and finally goes into solution. The mucilage layer gives no color with iodine and sulphuric acid, but stains red in an alcoholic solution of acid carmine. HESSE (9) measured the thickness of the membranes of the root hairs of many plants. He found that the thickness varied greatly with the family, but is nearly uniform within the family; that in any plant the thickness of any root hair mem-

brane varies with the medium; and that in *Rosa canina* growing in a dry situation the root hair has a stiff pointed apex and that the membrane is of lignin, but that in moist air no lignin is found.

LIQUID MEDIUM.—*Calcium nitrate.*—SCHWARZ (20) found that 15 per cent of calcium nitrate inhibited hair production. KRAUS (14) found roots richly haired in calcium nitrate.

Potassium nitrate—BARDELL (1) found that the root hairs on *Zea mais*, *Triticum vulgare*, *Avena sativa*, and *Tradescantia* sp. tend to decrease as the fractional solutions of KNO_3 increase in strength from 0.01 normal to 0.09.

Salt.—HILL (10) found that in *Salicornia* and *Suaeda* the root hairs can regulate their osmotic pressure in proportion to the osmotic pressure of the soil water.

Bogs.—TRANSEAU (24), working on bog plants, found that *Larix* roots when not surrounded by water develop root hairs abundantly. RIGG (19) used the development of the root hairs of *Tradescantia* as an indicator of the presence of toxins.

FOOD SUPPLY.—SCHWARZ (20) found that in reducing the food supply by removing portions of the endosperm the length of zone of root hairs decreased. SNOW (21) experimented with *Helianthus* and found that those with the most cotyledon remaining had the best formation of root hairs.

Investigation

This investigation was prompted by the fact that the presence or absence of root hairs is so often used as an indicator of the effect of changed external conditions. That the effect of the varying conditions might be known, it seemed well to try to determine the varying factors within the epidermal cell of the root, as well as the effect of the varying factors without and their reciprocal relations.

In order to picture as definitely as possible all the forces which might be affecting the epidermal cell, we may consider the average epidermal cell as having the form similar to fig. 1. Here it is seen that on four sides (*2*, *3*, *4*, *5*) the epidermal cell adjoins another epidermal cell, while on the inner side (*6*) it faces the wall

of a cortical cell, and on side *1* it is exposed directly to the varying external conditions.

In fig. 2, which is a median section of fig. 1, the following factors need to be considered: (1) *air medium*, (*a*) the physical character of walls *1, 2, 4, 6;* (*b*) the chemical nature of walls *1, 2, 4, 6;* (*c*) the osmotic pressure on both sides of walls *2, 4, 6,* and inside *1;* (2) *liquid medium*, (*a*) the physical character of walls *1, 2, 4, 6;* (*b*) the chemical nature of walls *1, 2, 4, 6;* (*c*) the osmotic pressure on both sides of walls *1, 2, 4, 6;* (3) *length of cells.*

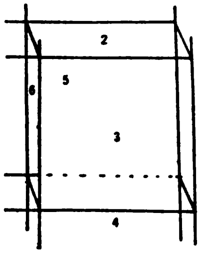

Fig. 1

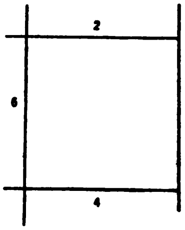

Fig. 2

Figs. 1, 2.—Fig. 1, form of an epidermal cell; fig. 2, median section of fig. 1.

A few measurements were made of the length of walls of cortical cells in comparison with the length of adjoining haired and unhaired epidermal cells. These were taken upon the same corn root, the haired epidermal cells being those just above water and the unhaired just below water, with the results shown in table II. These measurements, although few, indicate that there is no

TABLE II

Condition	Epidermal cell in mm.	Adjoining cortical cell in mm.
Haired	35	30
"	45	30
"	37	37
"	60	50
"	44	40
"	37	55
"	40	50
"	20	10
"	40	40
"	60	60
Unhaired	40	80
"	45–40	80
"	40	40
"	20	20
"	12	20
"	32	42

definite relation between the length of the cortical cell and the epidermal cell which decides whether the epidermal cell shall become extended into an outgrowth or not. Fig. 3 shows a haired cell longer than the adjoining cortical cell, and fig. 4 shows two short epidermal cells adjoining one cortical cell. These measurements are corroborated by observations made throughout the work, many forms and all possible variation in length of cortical and epidermal cells being found.

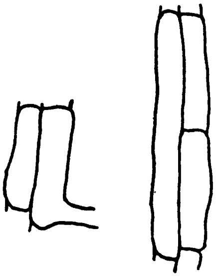

FIG. 3 FIG. 4

FIGS. 3, 4.—Fig. 3, haired cell longer than adjoining cortical cell; fig. 4, two short epidermal cells adjoining one cortical cell.

Position of nucleus.—The position of the nucleus has been found to have no relation whatever to the hair or to its initial formation. When the wall first swells, the entire wall bulges; later there is a greater swelling in one portion of it, the nucleus may not be near that portion; if the nucleus is near that portion, it may not follow the growth of the hair and be found near the tip of the hair, but remain in the base of the hair or in another part of the cell. There appears to be no relation between the position of the nucleus and the formation of the hair.

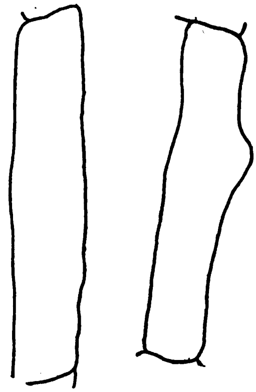

FIG. 5 FIG. 6

FIGS. 5, 6.—Fig. 5, bulging of entire wall; fig. 6, showing a narrowing of the swollen portion.

I. IN AIR MEDIUM

a) The physical character of walls 1, 2, 4, 6.—The first evidence of the formation of a root hair is the bulging of the entire wall *1*, as shown in fig. 5; then a portion of the wall stretches more than the rest and there is a narrowing of the swollen portion, as in fig. 6; but it may be noted that the base of a root hair is always greater in diameter than the rest of the hair, as in fig. 7. Seedlings of endive and lettuce demonstrate this fact. Branching of the hairs is common, indicating a difference in the physical character of the

wall *I*. Sometimes the branch will be of less diameter than the main hair, as in fig. 8; and again branches will be of equal diameter, as shown in fig. 9.

The stretched condition of the wall is evidenced by the fact that in some root hairs which are grown in moist air the membranes burst when the hairs are placed in water, or in a solution which has an osmotic pressure lower than that of the root hair cell, as shown in fig. 10; whereas plasmolysis occurs when the root hairs are placed in a solution of higher osmotic strength (fig. 11). The root hair remains normal when placed in a solution of an osmotic pressure equal to that of the cell content, as in fig. 12.

FIG. 7.—Base of the root hair greater in diameter than the rest of the hair.

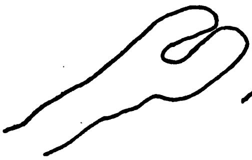

FIG. 8

FIG. 9

FIGS. 8, 9.—Fig. 8, branch of the root hair of less diameter than the main hair; fig. 9, branches of equal diameter.

FIG. 10

FIG. 11

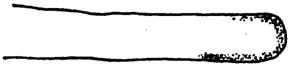

FIG. 12

FIGS. 10–12.—Fig. 10, root hair grown in moist air and placed in water, membrane burst and content escaped; fig. 11, root hair which has been in a solution of an osmotic pressure higher than that of the root hair; fig. 12, root hair placed in a solution of an osmotic pressure equal to that of the cell content.

The following forms when grown in moist air and then mounted in distilled water were found to burst immediately: alfalfa, barley, cabbage, mustard, *Nasturtium*, radish, *Xanthium*, and wheat. These when mounted in a solution of sucrose of an osmotic pressure equal to the osmotic pressure of the cell in question remain unbroken, whereas plasmolysis occurs if a solution of higher osmotic pressure is used. The cells burst at the tip and a part of the content of the cell is ejected. The nucleus is thrown

out if it happens to be at the tip of the root hair, but in case it is in or near the base of the cell it remains within the root hair. The break in the wall is immediately closed as the membrane springs back, and it is hard to detect the point of rupture except for the position of the escaped protoplasmic contents. The younger cells are more likely to burst than are the older ones. Fig. 13 (*a*) shows a radish root hair mounted in 0.24M sucrose solution; fig. 14 (*b*) is the same cell after being put in a 0.40M sucrose solution. It is to be noted that the walls corresponding to *2*, *4*, *6* are only very slightly changed, and wall *1* has decreased, thus showing that even in the same cell the physical condition of wall *1* is unlike that of walls *2*, *3*, *6*. In cells which do not burst when mounted in water, the surface of wall *1* is increased, as evidenced by a swollen tip which may or may not assume odd shapes. This indicates that wall *1* of these plants has a greater resistance, thereby indicating a variation in the epidermal cells of different plants, as well as a difference in wall *1* from walls *2*, *4*, *6*.

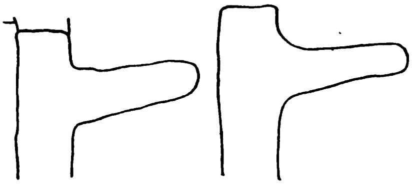

FIG. 13 FIG. 14

FIGS. 13, 14.—Fig. 13, root hair mounted in 0.24M sucrose; fig. 14, root hair after being placed in 0.40M sucrose.

It is frequently seen in many forms, for example, alfalfa, cabbage, and *Verbascum*, that short root hairs are mingled with long ones, giving the appearance of younger and older ones being together (fig. 15). The difference may be one of time of formation or of a variability in the growth of wall *1*; at least in these forms the length of the hairs is not a grading one as seen in the forms usually figured.

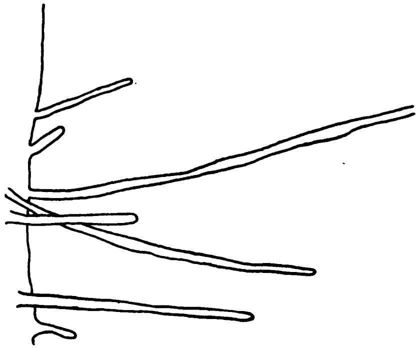

FIG. 15.—Short root hairs mingled with long ones.

b) The chemical nature of walls 1, 2, 4, 6.—It will be seen from table III that a majority of the forms investigated have a wall made up of two parts, an inner membrane which gives the cellulose reaction with 75 per cent H_2SO_4 and IKI, and an outer membrane which stains red with Ruthenium red, denoting pectin. That this membrane is calcium pectate is shown by the fact that on the addition of ammonium oxalate the membrane breaks down and calcium oxalate crystals are formed. It will be seen that some forms have a third membrane at the tip of the root hair. This membrane stains with all the callose stains, blue with aniline blue and resorsin blue, red with corallin soda. It will be noted that corn is the only form examined which has one membrane and that of cellulose.

TABLE III

Plant	Chemical nature of root hair membranes		
	Inner	Outer	At tip
Alfalfa..........	Thin cellulose	Thick pectin	
Amaranthus......	" "	" "	
Barley..........	" "	" "	
Corn...........	Thick "	....	
Corn salad.......	Thin "	Thick "	Callose
Cabbage........	" "	Thin "	
Daucus carota....	" "	" "	
Morning-glory....	" "	" "	
Mustard........	" "	" "	Callose
Nasturtium......	Thick "	Thick "	
Pea............	" "	" "	Callose
Sisymbrium......	Thin "	Thin "	
Tobacco.........	" "	Thick "	
Tradescantia.....	Thick "	Thin "	Callose
Vanilla..........	Thin "	" "	
Ceraptopteris.....	Thick "	" "	Callose

The walls *2*, *4*, *6*, and *1* are alike in that they all have an inner membrane of cellulose and an outer membrane of calcium pectate, but differ in that the calcium pectate membrane on wall *1* is thicker, and in some instances in the presence of callose at the tip of the hair. The calcium pectate membrane on side *1* is continuous with the middle lamella of calcium pectate of walls *2*, *4*, *6*. There is then a secretion of calcium pectate about the entire cell.

The relative thickness of the membranes having cellulose and pectin layers varies with the different plants, the cellulose layer being uniform over the hair, while the pectin is quite often thinner near the tip. Fig. 16 shows the tip of the root hair of *Nasturtium* with the two layers, the outer of pectin, and the inner of cellulose. Fig. 17 shows another *Nasturtium* root hair after it had been in ammonium oxalate; the calcium pectate has been changed to calcium oxalate and pectic acid. The calcium oxalate crystals are shown in the figure. The addition of Ruthenium red now gives no color except a faint tinge of pink about the crystals, due to the presence of pectic acid. Within the cell there are always masses of pectic substances; this is not calcium pectate, as it does not break down on the addition of ammonium oxalate, but still gives the color with Ruthenium red after treatment with ammonium oxalate.

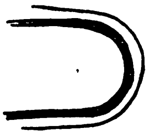

Fig. 16

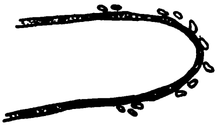

Fig. 17

Figs. 16, 17.—Two layers of the root hair membrane; fig. 17, *Nasturtium* root hair after being in ammonium oxalate.

Either of the membranes may be removed by treating the cell with their respective solvents. The calcium pectate membrane is soluble in 2 per cent HCl and 2 per cent KOH, the cellulose is soluble in copper-oxide-ammonia.

The fact that the soil particles are held to the hair by the transformation of the outer layer into mucilage has long been accepted, but what that mucilage is has not been known. In the case of corn growing in the soil or in quartz sand, the mucilage was found to be of cellulose, whereas in a *Coleus* root growing in the soil the soil particles are held to the hair by a pectin mucilage.

It has been known that the epidermal portion of the seedling from which hairs arise stains brown when put in a 2 per cent solution of potassium permanganate, and that the region just above that of the hairs is not colored by the solution. This has often been used to differentiate in a general way the stem and root regions of a seedling. The chemical nature of these walls was determined. The external wall of the epidermal cells above those forming root

hairs has without the calcium pectate membrane on side *1* of the epidermal cell a third thin layer which is of cutin. It stains red with Sudan III and is insoluble in 50 per cent chromic acid. This wall *1* of the epidermal cell of the stem is then chemically different and physically of greater rigidity.

c) The osmotic pressure on both sides of walls 2, 4, 6, and on inside of wall 1.—The cortical cell is always plasmolyzed by a solution of sucrose which is 0.02M–0.04M stronger than that which plasmolyzes the epidermal cell; this indicates that on the outside of the wall *6* there is a greater pressure than on the inside; whereas on the outside of *2* and *4* there is a pressure equal to that on the inside. On the outside of wall *1* there is a pressure of one atmosphere. The variation between the outside and inside pressures of wall *6* is small, but there is a great variation in the pressures upon the two sides of wall *1*. Table IV gives the osmotic pressure of the root hairs of different plants grown under similar conditions.

TABLE IV

Plant	Osmotic pressure of root hair	Plant	Osmotic pressure of root hair
Alfalfa	0.24M sucrose	Lettuce	0.30M "
Amaranthus	0.30M "	Mustard	0.22M "
Barley	0.40M "	Nasturtium	0.28M "
Corn	0.28M "	Pea	0.22M "
Corn salad	0.28M "	Radish	0.28M "
Cabbage	0.28M "	Sisymbrium	0.24M "
Celery	0.30M "	Sunflower	0.22M "
Daucus carota	0.24M "	Turnip	0.30M "
Endive	0.24M "		

It will be noted in table IV that all are plasmolyzed by a solution of sucrose between 0.22M–0.30M sucrose, except barley. This is the equivalent of 5.10–7.70 atmospheres. Two facts are indicated here: first, that in moist air there is a minimum of difference of 4 atmospheres between the pressure upon the inside and upon the outside of wall *1;* and second, that under similar conditions there is a remarkably slight variation in the osmotic pressure of root hairs of seedlings of different genera. Some roots respond more readily than others to the percentages of moisture present in the media. Cabbage, alfalfa, squash, and corn show a definite

response. The formation of the root hair is inhibited by removing the covers of the Petri dishes in which the seedlings are growing. Regions of no root hairs, corresponding to the growth made during the removal of the cover, followed in regions of hair formation at the period of its replacement.

A series of corn seedlings was grown in air of a known moisture content. This was controlled by percentage solutions of sulphuric acid. The corn was soaked for 24 hours and then hung on cork plates in bottles which were one-third filled with the varying solutions. The bottles, which were corked and paraffined, were all placed in a water bath kept at 24°C. The measurements in table V represent in each case the average of 5 sets.

TABLE V

Solution	Length of root in cm.	Diameter of root in mm.	Length of hair in mm.
Water	3.0	70	80
1 per cent H_2SO_4	1.0	64	70
2 " " "	2.0	65	70
3 " " "	3.0	63	66
4 " " "	2.0	60	50
5 " " "	1.5	70	70
6 " " "	1.5	60	50
7 " " "	1.5	55	50
8 " " "	1.0	65	60
9 " " "	1.5	66	41
10 " " "	1.0	64	23
11 " " "	1.0	61	24
12 " " "	1.0	70	10
13 " " "	1.0	70	10

Table V shows that the cutting down of the moisture content affects the length of the root hair more than the length of the root or the diameter.

2. IN LIQUID MEDIUM

a) *The physical character of the walls.*—There is a variation in the wall of the corn in different media. In moist air the wall is uniform; in water when formed the hair is thickened at the tip.

b) *The chemical nature of the walls.*—The chemical study of the walls is under investigation.

c) The osmotic pressure on both sides of 1, 2, 4, 6.—The only wall on which the osmotic pressure may be made to vary at will is on the outside of wall *1*. Radish seedlings were grown in sucrose solutions of increasing concentrations in order to vary the osmotic pressure on the outer wall. The results obtained as the average of several series are given in table VI.

TABLE VI

Grown in	Diameter root in 1/100 mm.	Length hair in 1/100 mm.	Osmotic pressure	Difference between osmotic pressure of root hair and medium
Air	60	....	28	28
Water	40	50	38	38
0.02 molecular sucrose	40	30	42	40
0.04 " "	40	20	42	38
0.06 " "	40	40	42	36
0.08 " "	40	50	49	32
0.10 " "	20	20	40	30
0.12 " "	40	60	42	30
0.14 " "	55	60	44	30
0.16 " "	30	40	48	32
0.18 " "	0	10	0	0
0.20 " "	0	20	0	0
0.22 " "	20	10	50	38
0.24 " "	30	10	65	41
0.26 " "	30	20	60	34
0.28 " "	50	20	65	37
0.30 " "	40	40	65	35
0.32 " "	30	10	0	0
0.34 " "	40	40	70	36
0.36 " "	15	10	75	39
0.38 " "	20	10	65	27
0.40 " "	30	20	0	0
0.42 " "	20	15	0	0
0.44 " "	50	15	70	36
0.48 " "	70	15	75	29
0.48 " "	0	0	0	0
0.52 " "	50	20	80	28
0.60 " "	50	0	90	30
0.65 " "	50	10	100	35

It is seen that as the concentration of the media increases the osmotic pressure of the root hair increases almost in direct proportion. Thus the root hair maintains an osmotic pressure from 4 to 6 atmospheres above that of the medium.

Differential media.—The effect of different media on the two sides of a seedling root was determined. Petri dishes were filled with the solutions, seeds were attached to the edge, and the roots

grew upon the solutions. The upper half of the root was in moist air and the lower half in the solutions. The roots grew straight, so that curvature could not account for the difference in the formation of the root hairs.

TABLE VII

Plant	Diameter of root in mm.	Length of hair (in mm.)	
		In air	In solution
Helianthus..........	8	8	1 (0.25M sucrose)
"	8	5	1 (0.20M "
"	5	5	1 (0.10M "
"	5	5	1 (0.05M "
Radish.............	5	8	2 (0.10M "

Discussion

The measurement of the length of cell walls as an indication of differential growth seems of little value unless the number of the cells in any region is taken into account. Since there is not an epidermal cell corresponding to each individual cortical cell, it would seem that the sum total of the epidermal cells should be known in order to determine differential elongation as a factor. So far as known, the measurements taken were of individual adjoining cortical and epidermal cells.

BARDELL's own measurements show that for corresponding epidermal and cortical cells there is no definite relation in length which would cause the epidermal cell to produce a root hair, as neither do the measurements found here on corn.

The factors which determine the initial swelling of the outer wall of the epidermal cell are of primary importance, while other factors come in which determine the later growth into a root hair.

It has been proved in root hairs that there are portions of wall *I* less resistant than others. The bursting of the root hair, the swelling, the branching, and the varying thickness of the membrane, all give evidence of this. The indications are that such weaker places exist in the membrane at the time of root hair formation, although this has not yet been proved definitely. These weaker regions would determine the region of the wall in which the locali-

zation of swelling takes place, which immediately follows the initial swelling of wall *1*.

The position of the nucleus can have nothing to do with the initial swelling, as the swelling is over the entire length of the wall *1*. Since the weaker places bear no relation to the position of the nucleus, the nuclear position can in no way affect the formation of the root hair.

In this discussion the second type given by LEAVITT is not considered, the type in which only specialized epidermal cells form root hairs. Preliminary observations indicate that the osmotic pressure of the short cells varies from that of the other cells, but there are doubtless other factors determining the hair formation.

Investigations so far indicate that there are two factors of importance in the initial formation of root hairs. One is the unequal pressure acting upon either side of wall *1*; the other is the variation in the physical character of the wall.

The difference in pressures on the two sides of walls *2*, *4*, *6* is so much less than the difference on the two sides of wall *1* that it is negligible. The osmotic pressure of the root hairs of the plants investigated when grown in moist air shows slight variation. In the plants examined the osmotic pressure approximates 5 atmospheres. The walls *2*, *4*, *6* have an opposing pressure equal to or greater than 5 atmospheres, but wall *1* must sustain a pressure of 4 atmospheres, for on this wall the internal pressure is opposed by only one atmosphere, when the root is grown in moist air. This pressure is sufficient to account for the initial swelling of wall *1*.

The result of increasing the osmotic pressure on the outside of wall *1* by growing radish seedlings in sucrose solutions of increasing osmotic value proved that the opposing pressures on either side of wall *1* still maintained a balance of at least 4 atmospheres in favor of an outward pressure.

Root hair formation is retarded when the moisture content of the air is decreased. This is shown by the experiments with seedlings grown in Petri dishes in alternating dry and moist air, and in those grown over sulphuric acid solutions. Reduced moisture affects the membranes by decreasing the extensibility of the walls due to an increase in the elasticity of the colloids. The osmotic